LASER MEDIATED RELEASE OF DYE FROM LIPOSOMES

David L. VanderMeulen

Chicago Institute for NeuroSurgery and NeuroResearch

Chicago, IL 60614

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Prabhakar Misra and Jason Michael

Howard University, Department of Physics and Astronomy

Washington, DC 20059

Kenneth G. Spears* and Mustafa Khoka

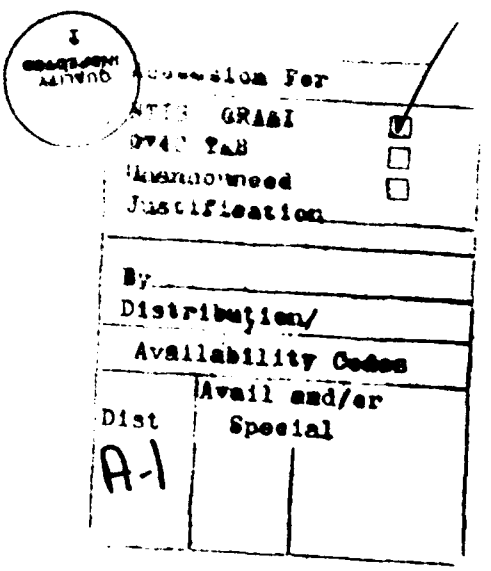
Northwestern University, Department of Chemistry

Evanston, IL 60208

*Author to whom correspondence should be addressed.

Abstract

Liposomes made from phospholipids and containing sulforhodamine dye (1-50 mM) have been irradiated with nanosecond and picosecond laser pulses. Individual liposomes were locally heated by laser absorption of dye dimers during a single laser pulse, and heating was sufficient to release the liposome contents. The extent of dye release produced by a single laser pulse was shown to be quantitatively dependent on several interdependent variables, including dye concentration, liposome size, laser excitation parameters and initial temperature of the dye-liposome system. Fluorescence lifetime data having three components have been obtained and analyzed in terms of three dye environments. Quantitative estimates support a photo-induced thermal mechanism for liposome lysis and release of its contents. These results may be useful for laser induced delivery of therapeutic agents or other applications of lasers in biological systems.



1. Introduction

In this work we provide mechanistic details of laser heating effects on model liposomes that have structural similarities to membranes of biological vesicles and cells. The application of laser photochemistry or laser heating to selective modification or inactivation of biological systems has been studied by Salet (1972) and Jay (1988).

Liposomes have been used as therapeutic agents and as research tools, and they have been employed both *in vitro* and *in vivo* as a drug and dye delivery vehicle as discussed by Zeimer et al.(1989), Khoobehi et al. (1989a) and Ostro (1987). Applications have included targeted delivery of antibiotic and antiviral drugs as discussed by Koff and Fidler (1985) and Price et al. (1989), and the selective modification or destruction of specific receptor and tumor sites as discussed by Poznansky and Juliano (1984). Genetic material has been delivered inside bacteria and cells using liposomes as carriers in the study of Straubinger and Papahadjopoulos (1983). Liposome carriers were used by Zeimer et al. (1988) in ophthalmology , by Adrian and Huang (1979) in enzyme replacement therapy and by Mannino and Gould-Fogerite (1988) in gene transfer studies. A number of applications as well as techniques for preparation have been described for unilamellar and multilamellar liposomes in the monographs of Gregoriadis (1984) and Ostro (1987).

Several non-laser approaches have been employed for efficient delivery of liposome contents to a specific cell site, which include among others, the study of Dijkstra et al. (1985) on the endocytosis of target cells, a study by Huang et al. (1987) on pH-sensitivity involving a lower interstitial pH, a study by Weinstein et al. (1980) on heat-sensitivity to temperatures above the transition temperature of the composite lipid(s) and the work of Pidgeon and Hunt (1987) on light-sensitivity. The light-sensitive techniques allow for controlled timing and differential dosage.

Photosensitizers can render an organism susceptible to damage by exposure to light, and organic dyes are commonly used as photosensitizers. The site of action of a particular photosensitizer within a biological system is largely governed by its intracellular behavior. For example, a lipid soluble photosensitizer would likely result in membrane damage. In the case of liposomes, the dye molecules can either be encapsulated within the interior cavity of the liposomes or be bound to their membrane bilayer. In the work of Grossweiner (1982) and Muller-Runkel et al. (1981) the well-known photosensitizer methylene blue was relatively ineffective in mediating photosensitized lysis of phosphatidylcholine liposomes without hydrodynamic agitation for oxygenation and long (ca. one hour) irraadiation periods.

A study by Magin and Niesman (1984) has shown that internalized substances can be released upon heating the liposome environment. The indirect heating method of microwave heating was used by Khoobehi et al. (1988) to study the eye. Indirect heating with lasers, such as Ar^+ laser heating of hemoglobin in the surrounding environment, has been studied by Khoobehi et al. (1990). Liposomes containing photosensitive dyes have been heated by laser absorption over a time long compared with thermal diffusion times of the liposome, and the heating of the extended liposome environment was used by Khoobehi et al. (1989b) to release the liposome contents at an appropriate site. This type of laser heating is directed, but not localized to an individual liposome. Heating of liposomes by laser excitation combines the effects of light and heat sensitivities, and provides a new method for controlled release of liposome contents.

In this study we show for the first time that the contents of liposomes with encapsulated dye can be released by direct, liposome localized heating, induced by single laser pulses of nanosecond or picosecond duration. We have demonstrated the dependence

of such dye release on laser pulse energy density, pulse width and sequence, dye concentration, liposome size and ambient temperature. Time-correlated photon counting and phase-resolved fluorescence spectroscopy techniques have been employed to determine multicomponent fluorescence lifetimes for dye-liposome complexes. Calculations based on an approximate quantitative model indicate a photo-induced thermal mechanism for release of liposome contents.

2. Materials and Methods

L-alpha-phosphatidylcholine (DPPC), dicetyl phosphate (DCP) and cholesterol were purchased from Sigma Chemical Co. Sulforhodamine 640 (equivalent to sulforhodamine 101) was obtained from the Exciton Chemical Co. The liposome-dye complexes were prepared by a modified solvent-rehydration technique as described by Szoka and Papahadjopoulos (1980). A mixture of DPPC/DCP (90:10, mol %) was dissolved in chloroform and methanol (90:10, vol %). Tris buffer (20 mM, pH 7.7) was used as a solvent to prepare equal volumes of the lipid and dye solutions. The lipid and dye solutions were mixed and rehydrated in a water bath at 55 ^{0}C. The mixture was subsequently cooled to room temperature and the excess dye unincorporated in the liposomes was removed with Sephadex G-25 columns (Pharmacia PD-10) equilibrated with Tris buffer. A Nicomp (Model 370) particle sizing system with a He-Ne laser was used to determine the size distributions of the liposomes by employing dynamic, quasi-elastic light scattering measurements. Software for data analysis provided both Gaussian and multimode distributions. Unfiltered liposomes were found to have an average size of about 2.2 μm. Polycarbonate membrane filters (Nucleopore Corp.) were useful for selecting liposomes of smaller diameters; whereas, low speed ($<10^3$ g) centrifugation

yielded liposomes of larger diameters. Typical standard deviations of the liposome sizes made by such techniques ranged from $\pm$ 0.5 μm for 2.0 μm liposomes to $\pm$ 1.0 μm for 4.0-5.0 μm liposomes.

A Perkin-Elmer (Model 330) UV-VIS Spectrophotometer was used to measure the absorption spectra of the liposome-dye system. For dilute solutions of sulforhodamine dye in the liposomes, the spectra consistently showed an absorption peak at 585 nm due to the monomer together with a shoulder at 545 nm due to the dimer.

Tris buffer (20 mM, pH 7.7) was used to dilute the liposome samples before laser excitation so that the absorbance at 532 nm was no larger than 5×10^{-2} for an irradiation path length of about 1 cm. For irradiation with a single 8 ns pulse from a frequency doubled output of a Q-switched Nd:YAG laser at 532 nm (Quanta Ray DCR1), 50 ul of sample was placed in a quartz capillary tube (i.d.= 2 mm; o.d.= 3 mm) and inserted into a custom-designed holder that fits into a standard 1 cm fluorescence cell. A telescopic combination of lenses focused the laser beam to a typical spot size of 0.25 cm diameter, while the energy per pulse was varied typically between 20 and 75 mJ. For irradiation with a single 25 ps pulse at 532 nm from a Q-switched Quantel/Continuum Corp Nd:YAG laser, 20 ul of sample was placed in a smaller 1 mm dia quartz capillary tube that was centered within the earlier 3 mm dia tube for stability. The ps pulse energy was varied in the range 0.75 to 7.80 mJ for a typical spot size of 0.16 cm. The filtered and diluted liposomes were irradiated with both 8 ns and 25 ps pulses along the long axis of the capillary tubes.

After pulsed laser irradiation, irradiated liposomes were analyzed by fluorescence. The capillary was placed transversely in the path of an excitation beam (585 nm) selected by a monochromator from a continuous white light source. Fluorescence from the

disrupted liposomes passed orthogonally through a second monochromator (620 nm) and was detected by a photomultiplier tube and picoammeter combination. The picoammeter output was input to a an Analog/Digital converter and microcomputer for signal processing and data analysis.

The fluorescence yield of sulforhodamine dye increases from about 1-2 % when encapsulated in liposomes at 20-50 mM concentration to almost 90 % in free solution following liposome breakage. For each irradiated sample, the percentage release of dye was quantitatively determined by comparing the fluorescence enhancement following liposome breakage with the maximum (100 %) release obtained after additional heating of the entire tube in a water bath maintained at temperatures above 55 ^{0}C. From Figure 1, the absorbance at 532 nm is coincidentally similar after thermal release so that relative yield measurements do not need a large absorbance correction. The maximum release from laser excitation was identical to that produced by addition of 0.05 % Triton X-100 surfactant to the liposome-dye complex.

Time-correlated photon counting experiments as described by O'Connor and Phillips (1984) were used to obtain fluorescence lifetimes for encapsulated and solution phase dye solutions. A frequency-doubled, CW mode-locked, Nd:YAG laser at 532 nm was used directly to excite fluorescence in some experiments and to pump a Spectra Physics (Model 3500) dye laser for tunable excitation with shorter pulse durations. Details of the apparatus have been described in prior work of Spears and Steinmetz (1985), except that in the present arrangement a faster ITT 4129 microchannel plate detector was used with an optimized constant fraction discriminator (Tennelec TC454). The 70 ps FWHM response function of the photon-counting system was obtained by detecting light from a scattering solution. Independent measurements were also performed at the Laboratory for

Fluorescence Dynamics at the University of Illinois, Urbana with the phase-resolved fluorescence method of Gratton et al. (1984), where we confirmed the slightly shorter lifetime of membrane associated dye than solution free dye. Membrane bound dye is the dye/lipid complex created by thermal release of liposome contents and removal of free solution dye by Sephadex chromatography. The cycle of heating and purification was repeated 2-3 times, and we have estimated that about 1 dye per 125 lipid molecules is bound in a dye/lipid complex, which is about 0.2-0.6% of the total dye concentration in the original liposome.

3. Results and Discussion

3.1 Photophysics and Energy Relaxation Kinetics

For dye concentrations above 1 mM, sulforhodamine encapsulated in liposomes shows a new absorption band at 545 nm due to dimerization. This absorption feature can be compared with the monomer peak at 585 nm in Figure 1. The dimer band exhibits strong quenching of fluorescence as described by Plant (1986) and Chen and Knutson (1988). The ratio of the dimer to monomer absorbance increases with rising internal dye concentrations from about 0.33 for dilute solutions to 0.7 for 1 mM sulforhodamine in liposomes. In Figure 2 we show that this ratio becomes essentially constant between 20 and 50 mM with a value of about 0.9. The relative constancy in the proportion of the dimers to the monomers over the range of encapsulated dye concentrations (20-50 mM) becomes an important parameter because the irradiation wavelength (532 nm) for the dye-liposome complexes falls in the dimer absorbance region with only slight overlap with the wings of the monomer band at 585 nm.

In liposomes having 20-50 mM dye, the fluorescence spectrum from dimer

excitation is identical to that from monomer excitation, and the quantum yield is 0.036 ±0.04 from monomer excitation at 585 nm. The spectrum and quantum yield from 532 nm excitation is as expected from the wings of the monomer band. As a result, most of the excitation energy at 532 nm is deactivated by non-radiative pathways of the dimer. Since both dimer and the monomer have low quantum yields, laser absorption is expected to produce localized heating in the liposomes following irradiation. The net efficiency of non-radiative conversion of absorbed laser excitation energy to thermal energy is estimated to be about 98 %.

Heat treatment above the critical gel-liquid crystal transition temperature (T_c = 41 ^{0}C) or treatment with a detergent brings about a dramatic disruption of the liposomes accompanied by a large increase in emission intensity. This sharp increase in fluorescence yield due to released dye provides a sensitive method for detecting the laser disruption of liposomes over a large dynamic range.

Fluorescence lifetime measurements were made to study the distribution of dye molecules in the bilayer membrane and within the interior of the liposome. Time-correlated photon counting with 585 nm excitation provided data on quenched (encapsulated) and unquenched (released dye). The free sulforhodamine dye in buffer solution had a fluorescence lifetime (T_f) of 4.2 ± 0.1 ns. The fluorescence time decays were complex for liposomes and required three-exponential least-squares fitting for the solutions containing 20, 35 and 50 mM sulforhodamine in 2.2 μm liposomes. A comparison of the percentages of the three lifetime components for 20, 35 and 50 mM dye in liposomes yields the relative contributions from weak membrane-bound complexes (longest lifetime T_1 component), partial-quenching (intermediate lifetime T_2) and full quenching (shortest lifetime T_3) processes involving the dye molecules. At higher dye

concentrations, the shortest lifetime component is the major fractional component. Table I summarizes the fluorescence lifetime data for 2.2 μm liposomes containing different concentrations of sulforhodamine dye. Similar lifetimes and percentages were obtained with 532 nm excitation. The errors in the lifetimes of the three component fit are larger than the single component fit of free dye. The longest component is estimated to be accurate to 5%, the middle component to 20% and the shortest component to about 20-50%. The fitting procedure provides a more accurate relative percentage for each component since the coefficient and lifetime tend to be correlated in such a way to preserve the total intensity of each time domain (product of lifetime and coefficient). The fluorescence lifetimes for free sulforhodamine dye in buffer and the membrane-bound component in liposomes (3.2 ns) were confirmed by independent measurements employing the phase-resolved fluorescence technique.

The interpretation of these liposome lifetimes is similar to the prior work of Chen and Knutson (1988), where the efficient quenching is assumed to be typical of Förster energy transfer. The assignment of the long lifetime component to membrane-bound dye molecules is consistent with lifetimes found for our thermal release procedure that isolated membrane bound dye. The intermediate component is assumed to be from those molecules not having geometric distances or orientations suitable for the most efficient energy transfer. From the fluorescence properties, we have inferred that dimer fluorescence is quenched by very fast radiationless transitions. In addition, the shortest fluorescence lifetime cannot be from dimer since from the lifetime data it would contribute about 10-12% of the total emission, which would have been detected as a unique spectral feature in the fluorescence spectrum. Furthermore, from the lifetime fitting we could infer that any dimer emission that was present may have been detectable in the lifetime

distribution if it had a lifetime greater than about 10 ps. Consequently, we believe that the dimer electronic state is rapidly quenched at picosecond or sub-picosecond rates. The observations discussed below on thermal breakage with picosecond pulses suggest a dimer relaxation rate faster than 1 ps.

3.2 Liposome Disruption

The extent of dye release caused by liposome disruption produced by a single laser pulse is quantitatively dependent on three major parameters, namely liposome size, dye concentration and laser energy density. Figure 3 displays the percentage release of liposome contents as a function of the energy density of an 8 ns laser pulse at 532 nm. The data is for three different sulforhodamine dye concentrations, 20, 35 and 50 mM. In all of these data the error bars represent standard deviations from 3 or more measurements. It is apparent from this graph that a single 8 ns laser pulse at 532 nm produces significant release of liposome contents for pulse energy densities exceeding 0.5 J/cm^2. Indeed, one obtains nearly 100 % release for 4.5 μm diameter liposomes containing 50 mM dye for pulsed energy density greater than 0.5 J/cm^2 (Figure 3). Similar measurements also were done with individual 532 nm pulses of 25 ps width. Figure 4 shows a comparison of the ps laser-activated release of sulforhodamine dye from the liposomes at three different concentrations (20, 30 and 50 mM). With increasing dye concentration, it is clear that there is an increase in the amount of laser energy deposition; i.e., for a given energy density of the laser pulse, the percentage release of dye was greater for higher dye concentration.

Diffusion loss of the thermal energy deposited by the laser excitation was probably significant during the 8 ns laser pulse. The effects of diffusion can be better understood

by assuming that that the rate of thermal diffusion in water at 25 ^{0}C has a maximum speed of 1.5 x 10^3 m/s for phonon propagation. Therefore, it is expected that diffusion of energy occurs over a distance of 12 μm in 8 ns. Thus, substantial diffusion losses could occur within the 8 ns laser pulse for micron or sub-micron liposomes, which is corroborated by our experimental data showing significant enhancement in efficiency of dye release as the average diameter of liposomes is increased from 1 to 5 μm. For example, with an incident 8 ns pulse of energy density 1.7 J/cm^2, 4.5 μm liposomes showed 85 % release of sulforhodamine as compared with 3.5 μm liposomes that yielded 68 % release. The effects of thermal diffusion would be expected to be substantially reduced for our experiments with picosecond laser pulses. Table II shows a comparison of the extent of liposome breakage using ns and ps laser pulses. For 20 mM sulforhodamine in 2 μm diameter liposomes, 29 % dye release was obtained with 25 ps pulses of energy density 0.32 J/cm^2 as compared with 8 ns pulses of energy density 1.60 J/cm^2. This five-fold reduction in energy density required for a 25 ps pulse to obtain a given percentage release of dye as compared with the 8 ns pulse was corroborated by measuring releases for 50 mM dye in 2 μm liposomes (Table II). These results qualitatively confirm the notion that picosecond pulses deposit thermal energy with negligible diffusion loss during the pulse, and thereby less total energy is required for achieving thermal release of liposome contents following pulsed laser irradiation.

Additional support for a heating mechanism being associated with dye release is provided by studying the laser-mediated disruption of liposomes for different initial temperatures of the dye-liposome system. Figures 5 and 6 summarize the effects of varying the ambient temperature of 2 μm and 4.5 μm size liposomes, respectively. These figures show the onset of sulforhodamine release as a function of the energy density of a

single 8 ns pulse. It is clear from the data that the percentage release of dye for a given energy density increases for larger initial temperatures. Such data further supports a heating mechanism as being responsible for liposome rupture, since breakage can occur only if the temperature exceeds the characteristic melting temperature of the bilayer membrane (T_c = 41 ^{0}C).

Effects of cholesterol on the phospholipid membrane system were also examined. Cholesterol is known to "stabilize" phospholipid membranes by producing broadened melting curves and shifting the effective melting temperature T_c towards a higher value with low (< 33 %) levels of incorporation. For excitation conditions that produced about 90 % release for DPPC/DCP membranes (88:12, mol/mol) from 16 mM sulforhodamine encapsulated liposomes, a 15 % cholesterol (mol/mol) incorporation in the bilayer reduced the amount of dye release to 30 %.

In order to understand why one hundred percent dye release is not obtained from liposomes exposed to a single laser pulse, 2.2 μm liposomes containing 20 mM sulforhodamine dye were irradiated with multiple, sequential pulses at nominal 1 second intervals to allow inter-pulse cooling. Two separate choices for the energy density of the 8 ns pulse, i.e. 0.8 J/cm^2 and 1.7 J/cm^2, provided two different starting values for the efficiency of dye release induced by a single pulse. It was observed that despite greater dye release with additional pulses, the percentage release tends to level off after about 5 pulses (Figure 7). Analysis of the data for sequential pulses indicated that the efficiency per pulse decreased from 25 % to 7 % for irradiation with 1.7 J/cm^2 pulses, and from 7.5 % to 2 % for 0.8 J/cm^2 pulses. Because a given liposome preparation exhibits a distribution of sizes, the preceding data can be interpreted (for a given dye concentration and specified laser energy density) in terms of thermal diffusion effects. We hypothesize

that the only population of liposomes undergoing breakage are those exceeding a certain minimum size, since thermal diffusion will cool smaller sizes. This will be true for nanosecond and picosecond pulses. However, the picosecond pulses will not have diffusion cooling during the excitation so that diffusion cooling competes with the kinetics of liposome breakage.

3.3 Model of Single Pulse Laser Breakage of Liposomes

A preliminary quantitative model based on the experimental data has been developed to explain the release of liposome contents following pulsed laser irradiation. For 532 nm laser excitation, each photon carries an energy of 3.7×10^{-19} J. Taking m to be the mass of intravesicular water, C_p = 4.18 J/(g)(^{0}C) as the specific heat of water and ΔT giving the change of temperature caused by photon absorption, the thermal energy deposited is

$E = m\ C_p\ \Delta T$. Assuming an absorption of one photon per dye molecule, there will be an estimated temperature rise $\Delta T = 2.7\ ^0C$ for a 2 μm diameter liposome containing m = 4.19×10^{-12} g of water. Thus, to raise the local temperature of the liposome by 27 ^{0}C to 52 ^{0}C (starting at 25 ^{0}C), which would be above the membrane transition temperature of T_c = 41 ^{0}C, there is a need for cycling about 10 photons per dye molecule in one laser pulse for 50 mM sulforhodamine in liposomes. A similar computation for 20 mM dye in liposomes requires cycling 25 photons per dye molecule.

Since excitation at 532 nm is within the dimer absorption band and the dimer is self-quenched at a rate that is nominally 1 ps or faster, multi-photon cycling per dye molecule is easily possible provided the laser power and the cross-section for absorption by the dye are adequately large. As a rough estimate of minimum power density to achieve

the maximum number of absorption cycles, one needs to saturate the absorption event during the lifetime of the transition. The nominal maximum number of absorption cycles is estimated by dividing the pulse duration by the relaxation rate; for example, in a 25 ps pulse with a 1 ps relaxation rate one could cycle 25 photons if the power is sufficient. For a dimer relaxation rate τ = 1 ps, using an absorption cross-section $\sigma = 1 \times 10^{-16}$ cm^2 and a photon energy E = 3.7×10^{-19} J, the anticipated saturation power density $E/\sigma\tau$ is 3.7×10^9 W/cm^2. Similarly, for the tail of the monomer absorption with a lifetime of 54 ps, the saturation power density would be 6.85×10^7 W/cm^2. A laser energy density of 1.0 J/cm^2 for a 8 ns pulse translates to a power density of 1.25×10^8 W/cm^2 which is too small to saturate the dimer absorption. For a 0.25 J/cm^2 and a 25 ps pulse the power density is 1×10^{10} W/cm^2 which is capable of saturating the dimer absorption if the lifetime is from 0.37 to 1 ps. In the case of the 8 ns pulse a large number of absorption cycles are not possible; however, an energy density of 1.0 J/cm^2 is sufficient to allow 25-30 absorption events per dye molecule with a mean time between events of 0.3 ns. Thermal diffusion during the 8 ns pulse reduces the heating effect per absorbed photon. The data in Figure 4 for the 25 ps pulse duration shows that efficient liposome breakage occurred for both 30 and 50 mM liposomes, which is consistent with having sufficient power density to take advantage of the fast dimer relaxation rate.

A more complete description of the dye-liposome system plus laser pulse duration requires a non-linear absorption/relaxation rate model that also includes thermal diffusion. Assuming a maximum energy diffusion rate given by phonon propagation with speed 1.5×10^5 cm/s and a representative liposome diameter of 3.0 μm, one estimates a maximum energy transport time of 2×10^{-9} s. Thus, an 8 ns pulse is long enough that a detailed diffusion model is required to estimate the heating efficiency. Even without a

very detailed mathematical model, our estimates have shown that a photo-induced heating mechanism is the most likely explanation for liposome breakage and release of contents.

The dye concentration and energy density variations in Figures 3 and 4 are qualitatively consistent with the above estimates of photon cycling. In particular, only for the 50 mM concentrations is the required number of cycles per dye molecule significantly less than the available cycles, which correlates with the observed high percentage release in one pulse. For lower concentrations more cycles are required and therefore the efficiency of release is less. For smaller liposomes greater diffusion losses occur. The sensitivity of the percentage release to starting temperature is consistent with the idea that the increased temperature from laser heating is not greatly above the transition temperature for the liposome. In Figure 3 the percent release versus energy density has a rate of rise to a saturation point that depends on the concentration. According to the prior analysis one expects a single sized liposome to have a specific threshold for breakage, and only in the case of 4.5 micron liposomes at 50 mM do we observe a fairly abrupt threshold for breakage. Since the laser parameters do not provide a great excess of energy deposition, the percent release should be very sensitive to the distribution of sizes for a liposome preparation. This was noted in Figure 7, which confirmed the idea of a threshold size. A more detailed kinetic model that also included size distributions would be required to fit the precise shapes found in Figures 3 and 4.

These experiments have demonstrated that dyes having strong absorption and fast relaxation rates can provide a means for very localized heating and controlled release of liposome contents with a single laser pulse. The localized heating and single pulse effect allows very selective laser action without injury to surrounding components that do not

have dye absorption. The power densities used in these experiments are quite large, but increased dye concentration or starting temperatures requiring less temperature rise can reduce the required power density. For focussed laser applications of a nominal cellular dimension of 10 μm one requires only 1 μJ to create power densities > 1 J/cm^2. For picosecond laser pulses, where diffusion is less of an issue, these power densities can be achieved in smaller focal spots approaching the diffraction limits, which could have applications requiring laser effects on small vesicles inside of cells.

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TABLE I

Fluorescence Lifetime Data for SR101 Liposomes

Dye Conc. (mM)	T_1 (ns)	T_2 (ns)	T_3 (ps)	C_1	C_2	C_3	P_1 (%)	P_2 (%)	P_3 (%)	χ^2
0.05	4.13	0.68	-	0.065	0.006	-	92	8	-	2.41
10.0	3.89	0.63	133	0.013	0.040	0.065	11	34	55	2.38
20.0	3.81	0.30	61	0.035	0.116	0.173	11	36	53	2.17
35.0	3.59	0.26	70	0.036	0.076	0.189	12	25	63	1.44
40.0	3.62	0.18	27	0.016	0.075	0.135	7	33	60	3.64
50.0	3.44	0.19	54	0.026	0.081	0.268	7	22	71	2.03

Notes: The errors in T_1, T_2, T_3 are estimated at 5%, 20% and 20-50%, respectively. The error in the percentages is about 10-20% for all values.

TABLE II

A Comparison of the Extent of Liposome Breakage with Nanosecond and Picosecond Pulses

Dye Concentration (mM)	Pulse-Width	Energy Density (J/cm^2)	% Release
20	8 ns	1.60	29
20	25 ps	0.32	29
50	8 ns	1.40	73
50	25 ps	0.28	73

FIGURE CAPTIONS

Figure 1. Absorption spectrum of 20 mM sulforhodamine encapsulated in liposomes (solid curve); spectrum of the dye after thermal release (dashed curve); spectrum of released dye normalized at the point of maximum absorbance (dash-dot curve).

Figure 2. Ratio of dimer to monomer absorption as a function of the encapsulated dye concentration in the liposome.

Figure 3. Effects of a single 8 ns laser pulse at 532 nm on release of sulforhodamine dye from liposomes as a function of energy density. Open and solid symbols refer to 2 μm and 4.5 μm diameter liposomes, respectively. (a) 20 mM (b) 35 mM and (c) 50 mM sulforhodamine dye

Figure 4. Effects of a single 25 ps laser pulse at 532 nm on release of sulforhodamine dye from 2.2 μm diameter liposomes as a function of energy density. (a) 20 mM (b) 30 mM and (c) 50 mM sulforhodamine dye

Figure 5. Effect of initial temperature on the single 8 ns laser pulse induced release of contents of 2 μm diameter liposomes with 50 mM sulforhodamine.

Figure 6. Effect of initial temperature on the single 8 ns laser pulse induced release of contents of 4.5 μm diameter liposomes with 50 mM sulforhodamine.

Figure 7. Effects of irradiating 2.2 μm diameter liposomes containing 20 mM sulforhodamine with multiple 8 ns laser pulses at 532 nm. Open and solid symbols refer to 0.8 J/cm^2 and 1.7 J/cm^2 pulses, respectively. There was about 1 sec delay between sequential pulses.

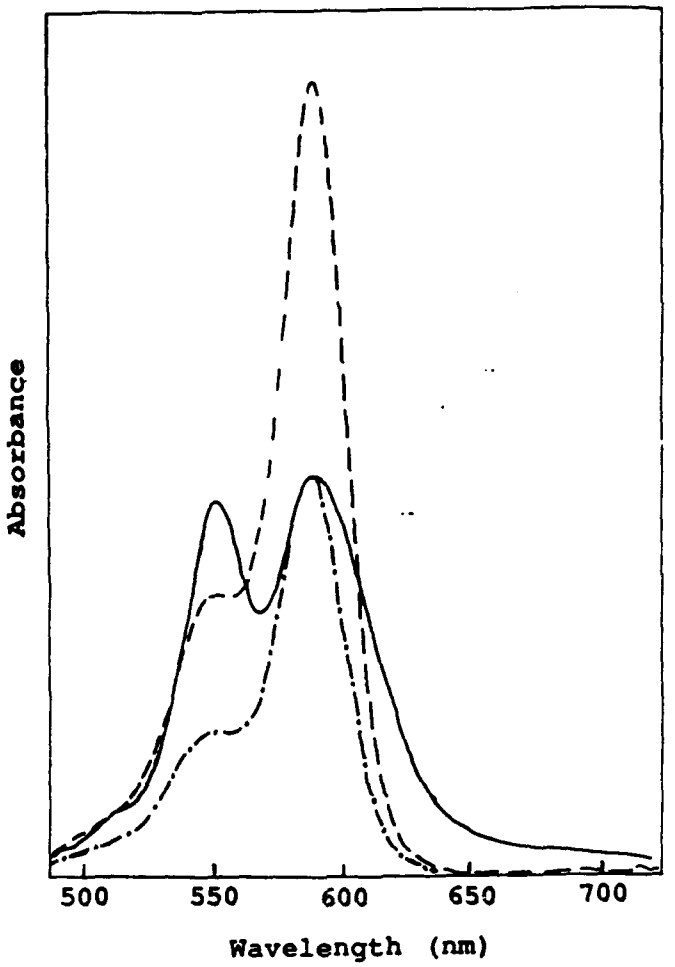

Absorbance
500
550
600
650
700
Wavelength (nm)

Fig 1

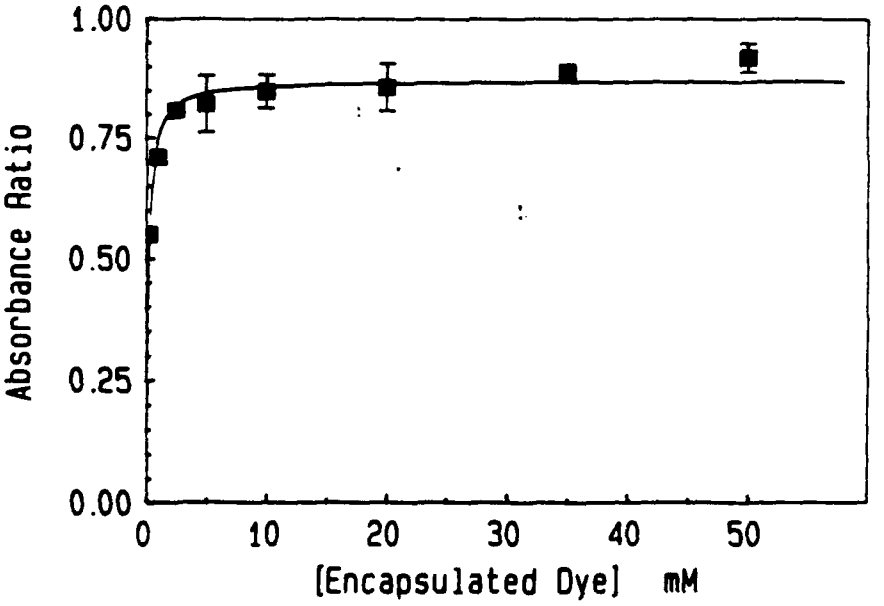

1.00
0.75
0.50
0.25
0.00
Absorbance Ratio
0
10
20
30
40
50
[Encapsulated Dye] mM

Fig 2

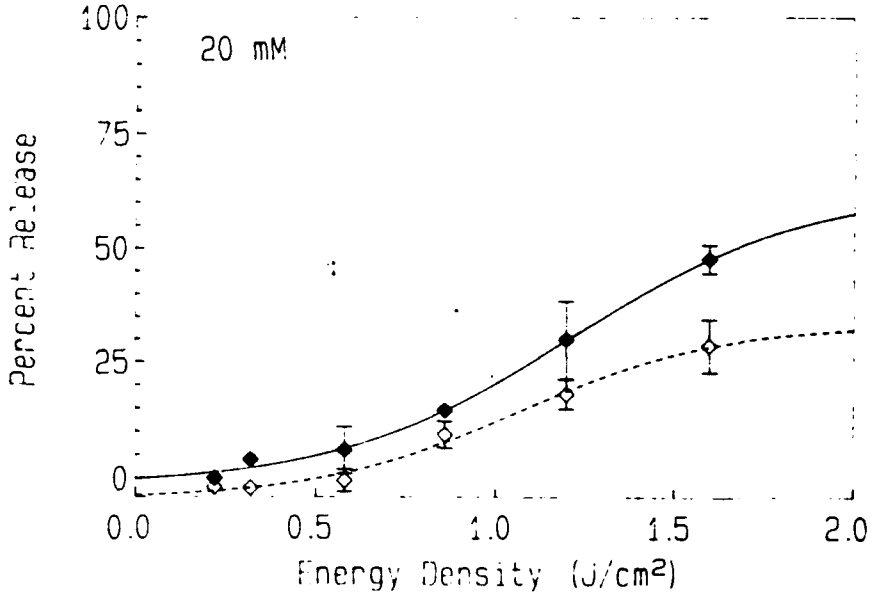


Fig 3a

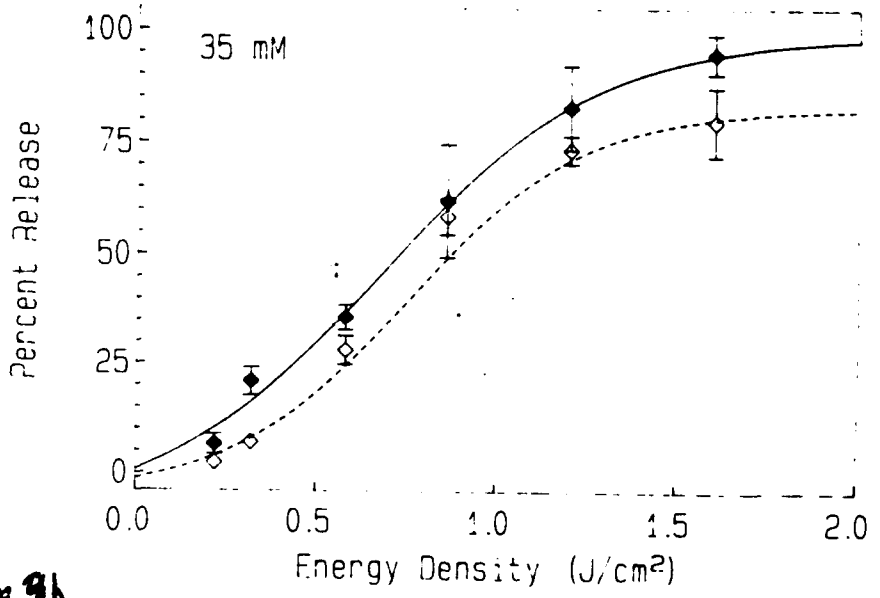
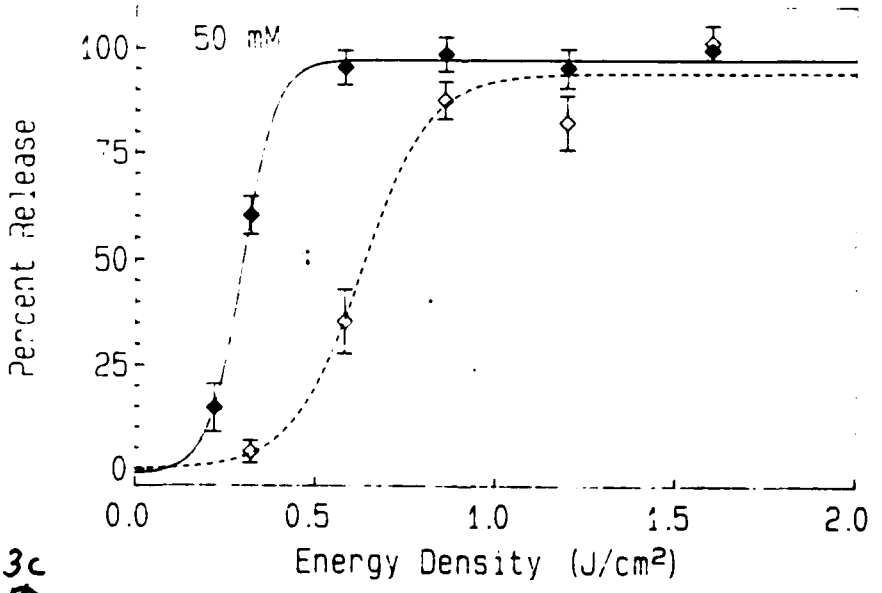


Fig 3b



3c

Fig 3c

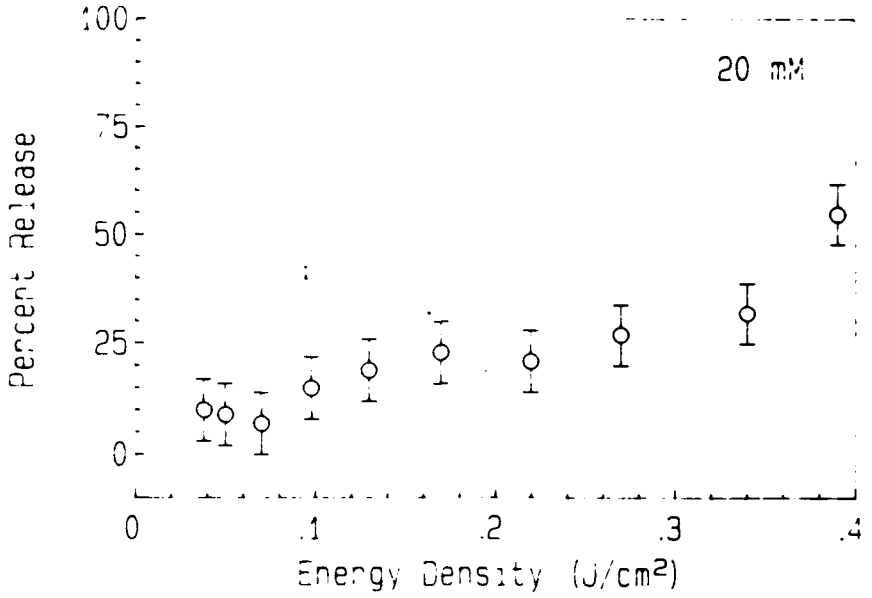


Fig 4a

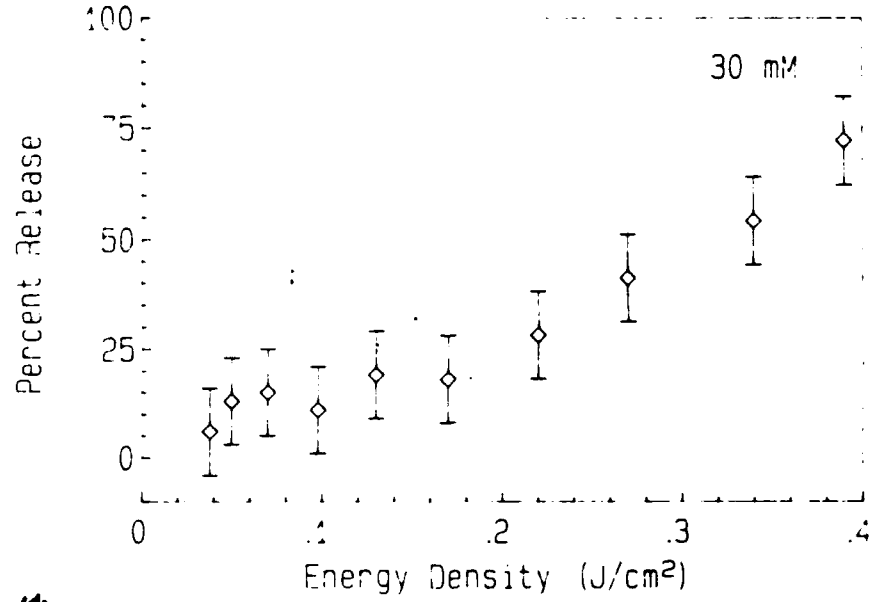


Fig 4b

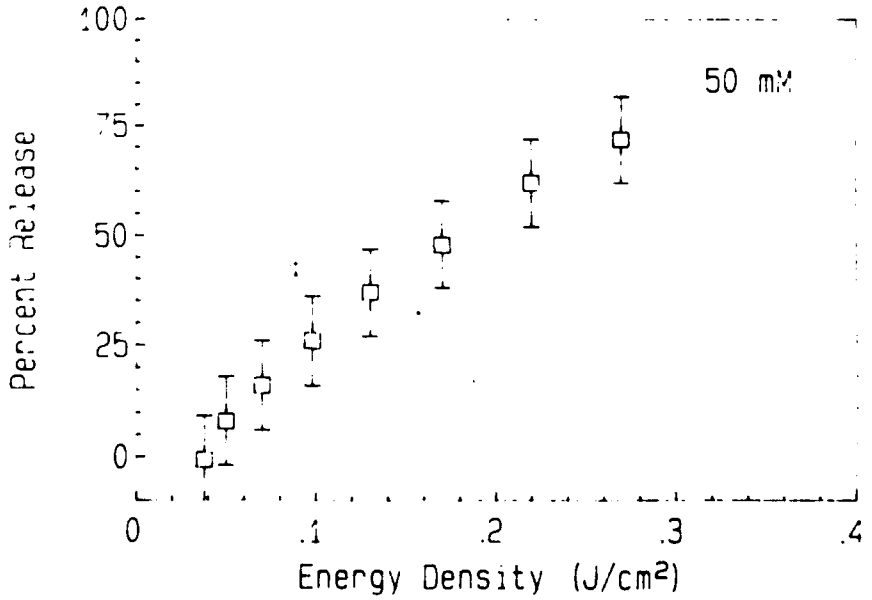


Fig 4c

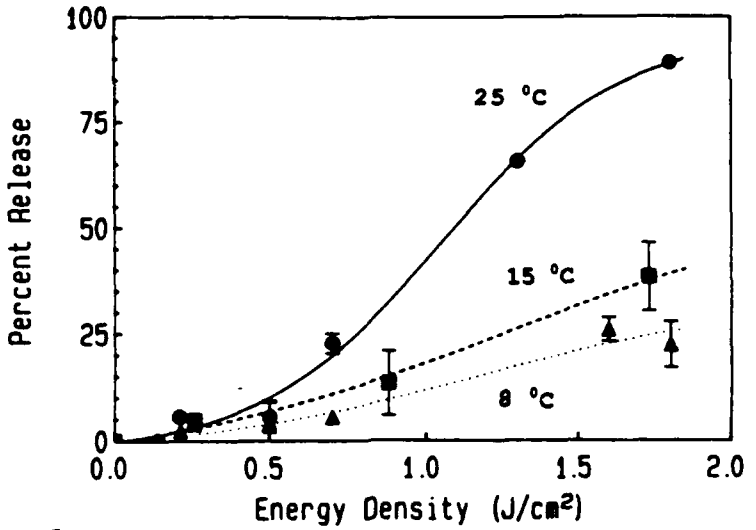


Fig 5

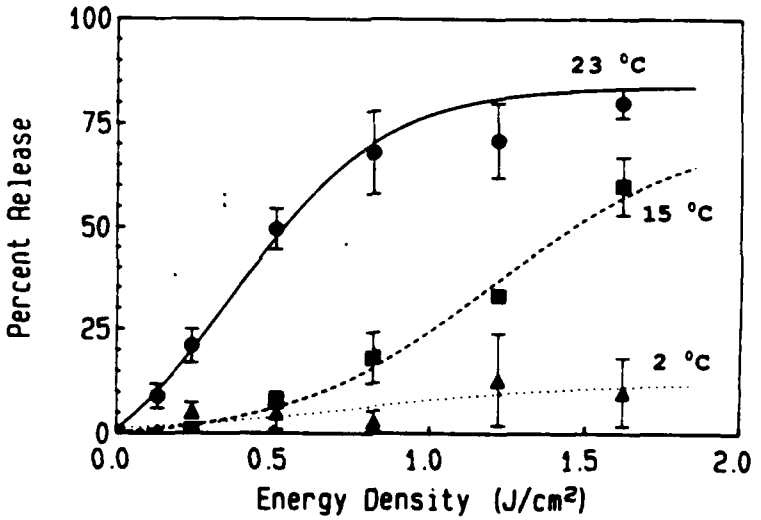


Fig 6

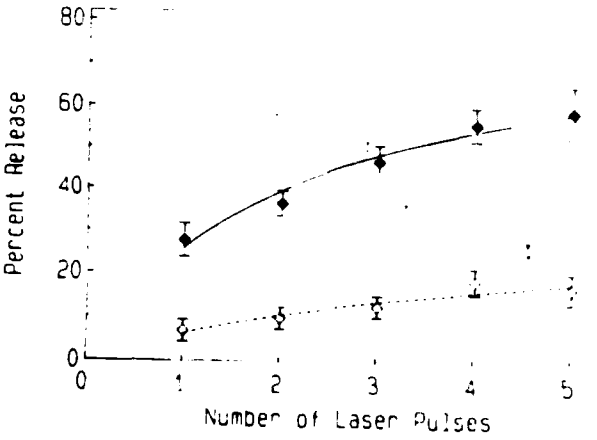


Fig. 7